REMARKS

Claims 1-3, 12, and 92-95 are pending. Claim 91 has been canceled, and the limitations of claim 91 were incorporated into claim 1. Claim 1 was also amended to further clarify the Akt gene as being wild type (e.g., as disclosed on page 13, line 30, of the specification). Claim 94 has been similarly amended. Claims 96 and 97 were added; these claims are supported by disclosure on page 3, lines 16-19; pag 14, line 3, to page 15, line 2; page 29, lines 20-23. No new matter has been added by this amendment.

37 U.S.C. § 112

Claims 1-3 and 12 remain rejected for overbreadth and lack of enablement. On page 2 of the Office Action, the Examiner states:

> while being enabling for regenerating myocardial tissue by local administration of isolated adult mesenchymal cells expressing an exogenous nucleic acid encoding an akt gene, and a growth factor gene; does not reasonably provide enablement for regenerating myocardial tissue by administering stem cells via any route (claims 94, 95, and it does not reasonably provide enablement for regeneratin myocardial tissue with mesenchymal stem cells expressing an exogenous nucleic acid encoding the broadly claimed injury-associated protein, or SDF-1 (paragraph spanning 2-3 of Office action)

Independent claims 1 and 94 have been amended to require local administration to a damaged portion of the myocardium.

Applicants note that claim 1 requires adult MSCs containing an Akt gene, whereas 92-95 require a second exogenous gene such as a growth factor. The specification is fully enabled for local administration of Akt-MSCs and contains ample data demonstrating regenerated myocardium after administration of MSCs containing an Akt gene (Figs. 10A-16B, and Example 3). For example, the specification at pages 35-37 discloses increased Akt-MSC survival, increased regeneration of myocardium, reduction in infarct volume, and normalization of cardiac function after local administration of Akt-MSCs to damaged myocardium. Thus, the full scope of claim 1 is enabled by the originally-file specification, and this rejection should be withdrawn.

Claims 93 and 95 further require that the Akt-MSCs contain an exogenous nucleic acid encoding SDF-1. With regard to these claims, the Examiner cited a 2003 Askari paper in Lancet to suggest unpredictability of these claims. On page 4, the Examiner states:

> This illustrated the state of the art and knowledge in the art was such unpredictable, knowing SDF-1 may be beneficial in treating condition of myocardial infarction does not establish the outcome is predictable, it is necessary to conduct further investigation to prove the hypothesis. It appears that Zhang et al was the first to provide evidence that supplying nucleic acid encoding SDF-1 is beneficial for myocardial repair. As to the genus of injury-associated polypeptides and using such for tissue regeneration, the only support in the specification is prophetic.

Applicants call the Examiner's attention to the Figures and portions of the specification cited above showing that MSCs containing an exogenous Akt gene confer benefit in treating myocardial infarction. The specification further teaches that those cells can also contain additional exogenous genes. Although the teaching of Akt-MSCs further comprising other exogenous genes is prophetic in that data using such cells is not included in the originally-filed specification, the specification provides ample guidance as to how to make and use such cells in fulfillment of the requirements of 112. The 2007 Zhang reference confirms that what was originally disclosed in Applicant's patent application works as Applicant described. Thus, this ground for rejection should also be withdrawn.

35 U.S.C. § 103

Claims 1-3 and 91 were rejected for obviousness over Matsui et al. in view of WO99/03973 ("Osiris").

As discussed in the previous Office Action, Matsui describes a gene therapy approach to cardiac dysfunction, not administration of cells (much less MSCs). In this reference, the author described injection adenoviral vector (containing a constitutively active Akt) into the myocardium followed by ligation of the left anterior descending coronary artery (LAD) forty-eight hours later. After 30 minutes the LAD ligature was released and reperfusion visually confirmed, and the rats were euthanized 24 hours after the ischemia. The Examiner is correct in stating that in this system, infarct size was reduced by 64% and apoptosis was reduced by 84%. (page 331, col. 1, of Matsui)

However, there are a number of fundamental differences between Applicant's claimed invention and Matsui's report. First, amended claim 1 now requires that the Akt gene be a wild type gene. In contrast, Matsui's results are based on a constitutively active Akt mutant. The wild type Akt is activated by hypoxia, oxidative stress, fluid shear, and inflammatory cytokines

(page13, lines 24-25, of the specification). This is a critical difference, as Applicants explain in the specification:

> Use of wild-type Akt, which was not constitutively expressed, but was activated when needed, protected cells from apoptosis, while avoiding the potential detrimental effects of constitutive activated -Akt expression. (page 13, lines 30-33, of the specification)

Second, Matsui administered his gene construct is administered to healthy heart tissue. The gene construct is administered to the heart, and then, 48 hours later, ischemia is induced by ligation of the LAD for a short period of time (30 minutes). Since the rats were killed at 24 hours post-ischemia, the data regarding infarct size and apoptosis only pertains to the 24 hour time point. Hypoxic cardiomyocytes expressing the control Akt construct was all dead at 24 hours (p332, col. 2 of Matsui). Applicants note that the cells expressing either type of Akt were cardiomyocytes, not stem cells. In contrast, claim 1 now requires that MSCs remain viable for 2 days, 3 days, and 3 weeks. Moreover, the data in the specification more closely mimics a clinical myocardial infarction scenario - LAD was ligated (to simulate an infarction) and then Akt-MSCs were injected sub-epicardially, normal sinus and hemostasis was obtained, the animals allowed to recover and hearts excised at 24, 72, and 3 weeks after injection. Given the significant differences in the test systems, the reduction in infarct volume and regeneration of lost myocardium demonstrated by Applicant's inventive method is indeed remarkable and surprising.

In response to Applicant's previous arguments, the Examiner has now substituted the Osiris reference ("which more clearly teaches that MSCs are capable of regenerating damaged cardiomyocytes *in vivo*") for Fukuda and Greenberger as a secondary reference. Although this reference does describe MSCs for the purpose of cardiac muscle regeneration, no demonstration or results are provided by this reference. The Examiner states:

> it would be obvious to one of ordinary skill in the art at the time the invention was made to modify the methods taught by *Matsui et al*, with either *WO 99/03973* or *Greenberger* and *Fukuda et al*, by administering mesenchymal stem cells expressing an exogenous Akt gene in place of the adenoviral vector as taught by Matsui et al with a reasonable expectation of success.

In rebuttal, Applicants point out that Matsui et al. used a constitutively active Akt mutant because "Akt activation reduces cardiomyocyte apoptosis…." (Abstract of Matsui et al.). The amended claims are distinguished from Matsui in that they require wild type Akt. The Matsui

vector was administered to cardiomyocytes, a mature and terminally differentiated cell type, that is prone to apoptosis under conditions of ischemia. In contrast, Applicants claim MSCs - mesenchymal stem cells - containing an exogenous nucleic acid encoding Akt. Stem cells are immature, undifferentiated cells, i.e., completely different in phenotype, behavior, and survival characteristics compared to cardiomyocytes. The Osiris reference describes use of MSCs for cardiac muscle regeneration and suggests genetic modification, but suggests only modification with genes "which express proteins of importance for the differentiation and/or maintenance of striated muscle cells". Examples given by the Osiris reference include growth factors such as TGF-P, IGF-1, and FGF; myogenic factors such as myoD, myogenin, Myf5; and MRF, transcription factors such as GATA-4; cytokines such as cardiotrophin; members of the neuregulin family such as neuregulin1, 2, or 3; homeobox genes such as Csx, tinman, NKx family; as factores that stimulate angiogenesis and revascularization such as VEGF. Genes that inhibit apoptosis are neither mentioned nor suggested. Thus, even if these references were properly combined, they might suggest a method using MSCs containing exogenous nucleic acids but not Akt. Akt does not fall into any of the categories suggested or contemplated, and there is not motivation in any of the cited reference to modify a MSC to contain an Akt gene.

In making the invention, Applicants specifically sought to overcome the deficiencies of early attempts to use MSCs (such as the Osiris reference) In the specification, Applicants review such early attempts by groups such as Osiris, Zhang et al. (2001; C52), Reineke et al.(C37, C38, C39), and Muller-Ehmsen et al. (C27) (all of record). For the Examiner's convenience, the relevant portions of Applicants' specification (spanning pages 28-29) is reproduced below:

> The group from Osiris Therapeutics has reported that putative MSCs derived from bone marrow that express C090 and proprietary markers SH-2 and SH-3, but not CD117 (c-kit) can differentiate into cardiac muscle *in vivo* However, implantation of as many as $6x10^7$ MSCs into infarcted porcine hearts yielded no improvement in cardiac function, because an estimated >99% of human bone marrow-derived MSCs die four days after transplantation into uninjured nude-mouse hearts.
>
> Although conceptually attractive, cell transplantation strategies to replace lost myocardium are limited by the inability to deliver large numbers of cells that resist pert-transplantation death to the ischemic myocardium. Reinecke *et al.* have demonstrated that nearly all donor adult rat cardiac myocytes are lost 24 hours after implantation into cry-injured adult rat hearts. Zhang *et al.* and Muller-Ehmsen *et al.* have shown that 30-60% of rat neonatal cardiac myocytes do not survive implantation into cryo-injured or uninjured hearts respectively; and fetal cardiac myocytes do not survive transplantation into infarcted hearts. Early attempts at preventing donor cell death have met with limited success.

Thus, the difficulties experienced for many years by many groups in the field have been overcome by Applicants' invention. Not only were Applicants' results unexpected and surprisingly beneficial, a long-felt and unfulfilled need has been met by Applicants' invention. These factors must be considered and weigh in favor of non-obviousness of the claimed invention.

Claim 12 and 92was rejected for obviousness over Matsui et al. in view of the Osiris reference in further view of Palasis, and claim 12 and 93-95 were rejected over Matsui et al. in view of the Osiris reference in further view of Pillarisetti et al. Applicants submit that the claim amendments and arguments presented above now distinguish the claims over these prior art references. Withdrawal of this rejection is respectfully requested.

CONCLUSION

Applicants submit herewith a Request for Continued Examination and a Petition for a Three-Month Extension of Time, along with the appropriate fees under 37 C.F.R. 1.17(a)(3). No additional fees are believed to be due in connection with this filing. If there are any questions, the Examiner is encouraged to contact the undersigned at the telephone number provided below.

The Commissioner is hereby authorized to credit any overpayment or charge any deficiencies to Deposit Account No. 50-0311 (Reference No. 18989-028).

Respectfully submitted,

/Reg. No. 41,874

Ingrid A. Beattie, Reg. No. 42,306
Attorney for Applicants
MINTZ, LEVIN, COHN, FERRIS,
GLOVSKY AND POPEO, P.C.
Tel: (617) 542-6000
Fax: (617) 542-2241
Customer No. 30623

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